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# SHORT-TERM SOCIAL MEMORY IN THE LABORATORY RAT: ITS SUSCEPTIBILITY TO DISTURBANCE

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24 Abstract

26 Adult rats presented with a juvenile conspecific for five minutes on two occasions, separated by a 15-min inter-exposure interval (IEI), investigated the reintroduced
28 juvenile significantly less in the second encounter. It is suggested that this was because the adult rats remembered the identity of the juvenile, because when a novel
30 juvenile was introduced for the second encounter, no such reduction in investigation was observed. When the rats were either handled, placed in a smaller, novel, cage, or
32 introduced to a new juvenile midway through the IEI, investigation of the reintroduced juvenile did not decrease. This indicated that memory of that juvenile
34 had been disrupted. However, a simple change of cage during the IEI had no disruptive effect on memory. These results suggest that routine husbandry procedures
36 can disrupt short-term social memory, which may lead to an increase in aggression due to a failure of recognition. This has implications for the welfare of captive social
38 animals.

40 Keywords: Social memory; Rat; Retroactive interference; Social recognition

## Introduction

Social memory, the ability of an animal to encode, retain, and refer to information concerning the identity of a conspecific over time, is likely to be crucial for the determination and maintenance of social structure in many animal species, particularly those living in small, stable social groups (e.g. Caldwell, 1985; Pagel and Dawkins, 1997). Yet, in contrast to the large amount of research on the abilities of non-human animals to discriminate between conspecifics (e.g. monkeys: Dasser, 1988; chickens: Bradshaw, 1991; rodents: Gheusi et al., 1994a; sheep: Kendrick et al., 1996; invertebrates: Karavanich and Atema, 1998), less attention has been directed towards social memory itself.

The disruption of cognitive function by stressful elements from both housing and husbandry systems could have potentially serious implications for the welfare of domesticates and captive wild animals (Mendl, in press). For example, if social memory is disrupted by husbandry procedures such as the removal and subsequent reintroduction of individuals from previously stable social groups, then the subsequent recognition failure may underlie the observed increase in aggression, and decline in welfare due to injury, which occurs when previously familiar animals are reintroduced (e.g. Ewbank and Meese, 1971). This raises questions about the potential stability of social memory in the face of interference resulting from environmental disturbances. Recent work on pigs has demonstrated that elements of common husbandry procedures can have a disruptive effect on the retention of a spatial learning task (Mendl et al., 1997; Laughlin et al., 1999). Here, we extend this work to investigate whether such procedures can interfere with social memory in rats.

Research investigating the effect of retroactive interference, the way in which an event introduced after an initial task can reduce subsequent performance of that task (e.g. Rodriguez et al., 1993), has revealed that spatial memory in rats appears resistant to disruption when the interpolated ('between-tests') event is different to the original learned task (e.g. Maki et al., 1979). However, if there is a large amount of interpolated experience, or if the interpolated event is very similar to the original task, it does appear that interference can occur (Roberts, 1981). Social memory studies have also found that interference can be induced by an interpolated event very similar to the original task. For example, when a new individual is introduced in the period during which another individual has to be remembered (e.g. Thor and Holloway, 1982; Dantzer et al., 1987).

In this study we therefore investigated whether social memory, like spatial memory, appears resistant to interference when the interpolated event is different to the original learned task. This information would thus allow us to compare between properties of the spatial and social memory systems. It would also allow us to investigate whether environmental disturbances, such as those commonly involved as part of husbandry or experimental procedures, can interfere with the social memory of laboratory rats. This study therefore has direct implications for the welfare of laboratory rats, and, if the rat is considered as a model species, may also have implications for the welfare of other captive social animals.

## General methods

## Subjects, housing and care

The subjects were unrelated male Lister Hooded rats (Harlan UK Limited), 12 at three months of age, and 10 at two months of age. We used two different ages of adult rats so that all the adults could undergo pre-experimental training simultaneously, but at testing all the rats would be the same age (3-months-old). Thirty two juvenile male Lister Hooded rats, 21 days old at the start of the experiment, were used as social stimuli. The time schedule was designed to ensure that the experiments were completed before the juveniles reached sexual maturation at around 50 days old (Wolfensohn and Lloyd, 1994). All the rats were housed individually in standard laboratory cages (33 X 50 X 21cm) with sawdust bedding and an enrichment toy. Food (Harlan Teklad Laboratory Diet) and water were provided ad libitum. The temperature of the experimental room was controlled (19°c ± 1), and maintained on a reverse dark-light cycle (light on from 1900-0700 hours), with observations carried out in the dark phase of the cycle. Red light (60 Watt) allowed the observer to see the rats. The rats were all handled for approximately 15 seconds each day, for one week prior to the start of the experiment. This was intended to familiarise the subjects to short bouts of handling, in order that transfer of animals between cages during experiments had minimal effect on behaviour. We used disposable gloves when handling the rats during the experiment, in order to reduce the chance of odour transfer.

## The social recognition test

This test, developed by Thor and Holloway (1982), is based on the natural

116 investigative behaviour of a rat, in which a rat shows a preference for investigating a novel conspecific over a familiar one (e.g. Carr et al., 1976). Social discrimination in
118 rats is regulated by the presence of olfactory cues (e.g. Sawyer et al., 1984), and investigation therefore takes the form of sniffing of the social stimulus, particularly
120 focusing on the ano-genital region (Carr et al., 1976). The test is based upon a comparison of behaviour, particularly investigation, between two exposures of the
122 same individual to a subject animal, separated by an inter-exposure interval (IEI). A decrease in investigation in the second exposure implies recognition of the individual.
124 No decrease suggests that the subject's social memory for that individual has decayed over the IEI, and is the response seen when a novel individual is introduced in the
126 second exposure. This demonstrates that any reduction in investigation is due to recognition of, and habituation to, a reintroduced stimulus, rather than a non-specific
128 decrease in the motivation to investigate a conspecific per se.

130 A benefit of this test is that it relies on spontaneous behaviour of the rat, but this also results in a less controlled experimental environment than those tests using
132 operant techniques (e.g. Gheusi et al., 1997). It is also unclear what cues the rats are remembering and using for the discrimination. In addition to olfactory cues, rats could
134 be discriminating on the basis of visual and auditory, especially ultrasonic, cues (e.g. Lore and Flannelly, 1977; Sales, 1991). Studies have indicated that it is unlikely,
136 however, that odour deposition, either by the adults on the juveniles, or by the juveniles in the home cages of the adults, influences the outcome of the social
138 recognition test (e.g. Sawyer et al., 1984; Perio et al., 1989).

140 Although this study was not designed to provoke aggression between animals,

there was a risk of aggression occurring during the social recognition test. To minimise this risk, juveniles were used as stimuli because previous studies had indicated that immature juveniles elicit little or no aggressive behaviour from adult rats (e.g. Thor, 1979). Other researchers state that although juveniles can be intimidated by adults, physical injury is rare (Lore and Flannelly, 1977). We found that aggression did occur, but at no point in this study was injury caused by mild aggression, defined as rolling and standing over the juvenile and/or pushing it away. A researcher was always present to ensure that if overt aggression occurred, e.g. biting, the encounter was abandoned immediately, with individuals separated before any injury was possible. Those juveniles who experienced overt aggression appeared to show no subsequent long-term effects, with normal behaviour and food/water consumption observed.

Behavioural observations

During each exposure of a juvenile to an adult subject rat, the total amount of investigation and mild aggression, in seconds, expressed by the subject during the course of the test period was continuously recorded by video camera and hand held event recorder (Psion Organiser II), using Noldus Observer software (Noldus Information Technology 1993). Investigation of the juvenile by the adult was defined as when the adult was nosing, grooming, sniffing, or following within one centimetre of the juvenile (Thor and Holloway, 1982). Mild aggression consisted of rolling and standing over the juvenile, and/or pushing it away.

Pre-experimental training

The fact that, following a 5-min initial exposure, a male adult rat typically recognises a juvenile after an IEI of 30min, but fails to recognise a juvenile after 120min, is often taken as a standard measure of social recognition (e.g. Dantzer et al., 1987; Gheusi et al., 1994b). But there are instances where recognition does not appear to occur after a 30-min IEI, notably in sexually inexperienced, young (3-months-old) adult males (e.g. Hlinak and Krejci, 1991; Engelmann et al., 1995). For this reason we made preliminary observations during pre-experimental training, to ensure that the initial exposure and IEI length selected for the disturbance experiments allowed successful recognition.

This also allowed us to remove not only any overtly aggressive rats, but those which failed to investigate the juveniles reliably. The pre-experimental training session consisted of an exposure to the same juvenile, once a day for four consecutive days. Half the adults received exposures of 5-min duration, the remainder received exposures of 15min. We introduced a novel juvenile on the fifth day, followed by re-exposure to the original juvenile on the sixth day. A second exposure on the sixth day of the same original juvenile to the adults, after a short inter-exposure interval of either 15 or 25 minutes, was designed to reveal which combination of initial exposure and inter-exposure interval lengths was most suitable for use in the main experiments reported here.

Experiment one

This experiment was designed to investigate the effect of introducing different potentially disturbing environmental stimuli, (see Table 1), on the social memory of laboratory rats.

*Table One*

These treatments, excluding the control treatment (treatment A), were selected as being representative of elements of commonly occurring husbandry/experimental procedures. If these treatments were found to have a disruptive effect on the social memory of laboratory rats, then this could have important implications for the welfare of these animals, and the accuracy of some experimental research (see Introduction).

## Method

Two of the 12 potential subjects (3-months-old) for this experiment were excluded due to inappropriate behaviour during pre-experimental training. One was too aggressive, and the other investigated the juvenile stimulus unreliably, displaying apparently submissive behaviour. We therefore used 10 rats in two replicates (N=5 per replicate).

The results obtained from pre-experimental training suggested the chosen timing regime. The experimental procedure consisted of an initial 5-min exposure of a particular juvenile to a resident adult e.g. "$A^1$", followed by a 15-min IEI before reintroduction of the same juvenile, "$A^2$", (see Fig. 1). After 5min of the IEI, each adult was exposed to its particular treatment for a 5-min period. Each adult

experienced the procedure once on each experimental day. The effect of treatment order on the behaviour of the subject animals was taken into account by using a Latin square design (5 X 5). This experimental design also ensured that the number of animals used was minimised (Still, 1982). The two replicates were tested on alternate days such that each rat received all five of the treatments, one every other day, the order determined by the Latin square. All exposures to the juvenile stimuli took place in the home cage of the subjects. Sixteen juveniles were required in total, with adults encountering a different juvenile on all five test days, and no juvenile used more than once for the same treatment. All the juvenile stimuli were entirely novel to the subjects.

*Figure One*

Investigation and mildly aggressive behaviour were recorded for analysis (see earlier). The novel environments (treatments D & E) were wiped down with a mild disinfectant before the introduction of the adult rats in order to reduce the possible influence of lingering odours.

Data analysis

Data ($N$=10) were assessed for normality and homogeneity of variance, and those that failed to meet these criteria underwent logarithmic transformation. Pre-treatment behaviour was analysed first, to ensure that there were no pre-treatment differences between the treatment groups. The difference between pre-treatment and post-treatment behaviour was then calculated (first - second exposure) and analysed.

The difference in the total amount of behaviour displayed during each of the two exposures to the juvenile was used as a measure of behavioural change, because other measures, such as ratios (e.g. Perio et al., 1989), can fail to uncover differences in the absolute size of response. The data were analysed initially using a GLM for repeated measures, with treatment (1-5) and pre-experimental training experience (5 or 15min) as factors. Paired t-tests were used to investigate specific differences within individual treatments. The statistical package used was Minitab (Minitab Inc. 1996).

## Results

There was no difference between the five treatments in the amount of either investigation ($F_{4,32}$=1.39, N.S.) or mild aggression ($F_{4,32}$=1.11, N.S.) expressed pre-treatment. There was also no effect of previous experience, or any interaction between experience and treatment, for either investigation or mild aggression. When the difference in behaviour between the two exposures was analysed, a significant effect of treatment on investigation was found ($F_{4,32}$=2.75, $P$<0.05), but no effect on mild aggression ($F_{4,32}$=1.42, N.S.). Again, there was no effect of previous experience, or interaction between experience and treatment, for either investigation of mild aggression.

*Figure Two*

Paired t-tests were used in order to ascertain in what way each of the treatments were influencing the change in investigative behaviour between the first and second exposures (see Fig. 2). No significant change in investigation was

observed between exposures for treatments B (handling), C (novel conspecific) or D (small novel environment). But a significant decrease in investigation was seen for both treatment A (control) ($T$=2.98, $N$=9, $P$<0.05) and treatment E (novel environment) ($T$=4.19, $N$=9, $P$<0.001). It therefore seems that the treatments had different effects on the investigative behaviour of the subject rats.

## Discussion

These results suggest that during both the control and novel environment treatments the rats retained a memory of the juvenile conspecific over the 15-min inter-exposure interval. The introduction of the rats to a novel environment of similar size to their home cage did not appear, therefore, to disrupt their social memory. The remaining treatments, however, did appear to disrupt social memory. The apparent interference effect of a novel conspecific confirmed the results of previous studies (Thor and Holloway, 1982; Dantzer et al., 1987). There appeared to be no effect of different pre-experimental training experience on the subsequent behaviour of the adult subjects.

The introduction of a novel conspecific is the treatment most closely linked with the ‘learning task’ itself, and may directly compete with information stored about the original juvenile. Differences in the effect on behaviour between the two novel environment treatments suggest that the increased confinement of the smaller environment was likely to have been the cause of the observed disruption of social memory. The subject rats had experienced only brief bouts of handling prior to the implementation of the handling treatment. It is therefore possible that an increase in

290 stress caused by this treatment resulted in a disruption of social memory. It should be noted that because each treatment was implemented half way through the IEI, they
292 might have affected either memory consolidation or recall (see Mendl, in press).

294 An alternative explanation for the observed results is that the treatments caused the investigative behaviour of the rats in the second exposure to rise above the
296 expected habituated levels despite an intact social memory, possibly by affecting non-specific states of arousal or motivation. Attempting to determine whether treatments
298 have disrupted social memory or provoked an increase in motivation or arousal is problematic. For example, one might expect an aroused adult to investigate a novel
300 juvenile introduced for the second exposure significantly more than if the original juvenile had been reintroduced. But even if this was the case it may still not prove
302 conclusively that the treatment was having an arousing effect, as significant increases in investigation can occur upon the introduction of a novel juvenile even when no
304 treatment has occurred (e.g. Sekiguchi et al., 1991). Because of this difficulty, we focused our attention on treatment E (novel environment), which had not appeared to
306 disrupt social memory.

308 Experiment two

310 This experiment was designed to confirm the findings of experiment one, and investigate the effect of treatment disturbance on the social memory of the subject rats
312 by comparing behaviour following reintroduction of the original juvenile stimulus ($A^2$) with that following the introduction of a novel juvenile (B) (see Fig. 3). In the
314 previous experiment we observed that the removal of an adult rat from its home cage

to a large novel environment apparently failed to disrupt the social memory of that rat for a juvenile conspecific, allowing recognition. Alternative explanations for this result are that the juvenile was not actually recognised, but that investigation was reduced either because of the adult habituating to the experimental procedure, or the treatment itself acting to suppress behaviour. Both of these explanations also predict a similar reduction in investigative behaviour if a novel juvenile is introduced for the second exposure, whereas an increase in investigation might be expected if no disruption to social memory is occurring. The same technique has been used to distinguish between non-specific and specific effects of drug treatments on social memory (e.g. Perio et al., 1989; Gheusi et al., 1994b).

*Figure Three*

In this experiment, four treatments were used (see Table 2).

*Table Two*

Method

Two of the 10 rats, previously 2-months-old, were excluded because of overt aggression during pre-experimental training, so eight rats, now three months old, were used. Two replicates (N=4) of a Latin square design (4 X 4) were used to assign treatment order across time, and 16 juveniles were used in total. As before, all juveniles were introduced only twice a day, were used for different treatments each day, and were entirely novel to the particular adult to whom they were introduced.

The two replicates were tested on alternate days such that each rat received all four of the treatments, one every other day, with treatment order determined by the Latin square design. The novel environment was cleaned with a mild disinfectant between uses. Investigation of, and mild aggression directed towards, the juvenile was recorded.

Data analysis

Data ($N$=8) were treated as before (see experiment one).

Results

No significant difference between the four treatments types in the relative amounts of either investigation or mild aggression was observed pre-treatment. There was also no effect of previous experience, or any interaction between experience and treatment, for either investigation or mild aggression.

*Figure Four and Figure Five*

Paired t-tests were used to investigate exactly how the different treatments each affected investigation and mild aggression. Significant changes in the amount of investigation between the two exposures to the juvenile stimuli were discovered for three out of the four treatments (see Fig. 4). Treatment one (no interference, same juvenile reintroduced) ($T$=4.00, $N$=7, $P$<0.01) and treatment two (novel environment, same juvenile reintroduced) ($T$=2.76, $N$=7, $P$<0.05) both showed significant

reductions in the amount of investigation between exposures, whereas treatment three (no interference, novel juvenile introduced) displayed a significant increase in investigative behaviour ($T$=-2.83, $N$=7, $P$<0.05). Investigation also increased in treatment four (novel environment, novel juvenile introduced), and this increase was nearly significant ($T$=-2.24, $N$=7, $P$=0.06). The only significant change in the amount of mild aggression observed, was an increase in mild aggression following the implementation of treatment one (no interference, same juvenile reintroduced) ($T$=-2.43, $N$=7, $P$<0.05) (see Fig.5).

## Discussion

These results confirmed the findings of experiment one in which treatments A (no interference, same juvenile reintroduced) and E (novel environment, same juvenile reintroduced), showed similar decreases in investigation, suggesting that, for both those treatments, the adult rats recognised the juveniles reintroduced into their home cages.

The results also demonstrate that any observed reduction in the amount of investigation of the juvenile by the adult rat was due primarily to the habituation of the subject to the stimulus, i.e. recognition, rather than because of any behaviour suppressing property of the treatment, or general habituation to the experimental procedure. This emphasises the importance of introducing a novel juvenile as a control to distinguish between specific and non-specific effects of treatment (e.g. Perio et al., 1989; Gheusi et al., 1994b).

## General discussion

The results of experiment one indicate that the implementation of some elements of common husbandry/experimental procedures appears to be sufficient to disrupt the short-term social memory of an adult laboratory rat, although we are as yet unable to completely rule out the possible effects of these environmental stimuli on non-specific states of arousal or motivation. Removal to a large novel environment did not appear to interfere with the social memory of the adult rats, and experiment two confirmed that any reduction in the total amount of observed investigation was unlikely to be due to behavioural suppression or motivational change.

Other researchers have observed anecdotally that the way animals are handled can be important for minimising variation in baseline investigation times (Dantzer et al., 1987), and that removal from the home cage between exposures fails to interfere with subsequent recognition (Perio et al., 1989). This paper, in contrast, specifically investigated the effects of mild environmental stimuli on social memory, and therefore has implications for the use of the social recognition test in research. Experiments that apparently indicate recognition failure may actually result as a side effect of the experimental technique itself, i.e. excessive handling, rather than because of the specific treatment. The influence of these external factors should therefore be taken into account. The findings of this experiment also reflect work on the disruption of spatial memory in pigs (e.g. Mendl et al., 1997; Laughlin et al., 1999) and demonstrate that, at least in the short term, social memory in rats can be disrupted. The exact way in which the memory has been disturbed is more open to question. The introduction of an environmental stimulus shortly after the learning task may disrupt

memory formation, or, once the social memory for a particular individual has been formed, the introduction of an environmental stimulus may act to block attempts to retrieve the retained memory (Mendl, in press).

When compared to the spatial memory of rats, social memory appears more susceptible to disruption, even by relatively mild environmental stimuli. There may be species specific reasons for this apparent discrepancy. For a group living animal like the wild rat (Barnett 1963) it is perhaps only worth forming a lasting memory of another individual if there is a high probability of repeat encounters with the same individual over a short space of time (Caldwell 1985), particularly if this recognition forms the basis for a dominance hierarchy (Pagel & Dawkins 1997). This may be difficult for a rat to evaluate on a first encounter, so it may not be cost-effective to keep forming a memory of a new individual on the first meeting, as that same individual may never be encountered again. There is strong biological foundation for the notion that maintenance of accurate memories requires substantial resource expenditure (Dukas 1998). In direct contrast to social memory, spatial memory needs to be more immediately resistant to disturbance over the short-term. The location of a food source or a potential nest site, and routes to and away from familiar areas, must instantly be stored in memory otherwise the animal could starve or become lost. There is therefore potentially a far greater requirement for spatial memory to be remembered in the short-term because of the greater cost to the animal of memory failure. One could therefore predict that spatial memory is less susceptible to disturbance over short-term than social memory, as found, but that social memory will become increasingly resistant to disruption after long term formation. One might also predict that those individuals who ‘mean’ more to a specific individual will be remembered

faster and be more resistant to disruption than those with little, or no, meaning, i.e. the referent of the social stimulus may have a direct effect on the way it is processed.

## Conclusions

These results could have implications for the welfare of both laboratory rats and other captive social animals. The removal of an individual rat from a social group followed by the inadvertent introduction of a potentially disruptive environmental stimulus, may mean that upon return to its social group the rat is unable to recognise its former companions, resulting in an increase in aggression and corresponding reduction in welfare. However, this study looked only at the effects of environmental disturbance on short-term social memory. It is likely that with a longer initial exposure, social memory will become more resistant to disruption. It is therefore essential that the current work is followed up by investigation into more long-term social memory.

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546 Table 1: Descriptions of the different treatments received between exposures

| | |
|---|---|
| Treatment A | No Interference (control): Subject remains in home cage between introductions of juvenile |
| Treatment B | Handling: Subject is handled in its home cage for five minutes between juvenile introductions, consisting of being picked up every 15 seconds for five seconds |
| Treatment C | Novel Juvenile: A novel juvenile is introduced to the home cage of the subject for five minutes between exposures of the original juvenile |
| Treatment D | Small novel environment: Subject was introduced to a small novel environment measuring 30 X 13 X 11cm with a plastic floor surface for 5 minutes between introductions of the juvenile |
| Treatment E | Novel Environment: Subject was introduced to a novel environment measuring 33 X 50 X 21cm (same size as the home cage) with a metal wire floor surface for five minutes between introductions of the juvenile |

548 Table 2: Descriptions of different treatments received between exposures

| | |
|---|---|
| Treatment 1 | No Interference (Same): Subject remains in home cage between introductions of same juvenile. |
| Treatment 2 | Novel Environment (Same): Subject was introduced to a novel environment measuring 33 X 50 X 21cm with a metal wire floor surface for five minutes between introductions of the same juvenile. |
| Treatment 3 | No Interference (Novel): Subject remains in home cage between introductions of novel juveniles. |
| Treatment 4 | Novel Environment (Novel): Subject was introduced to a novel environment measuring 33 X 50 X 21cm with a metal wire floor surface for five minutes between introductions of the novel juveniles. |

Figure One

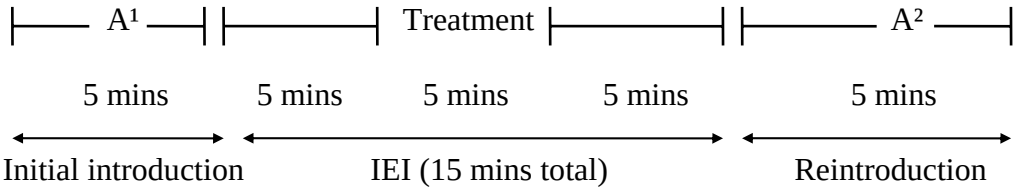

A¹
Treatment
A²
5 mins
5 mins
5 mins
5 mins
5 mins
Initial introduction
IEI (15 mins total)
Reintroduction

Figure Three

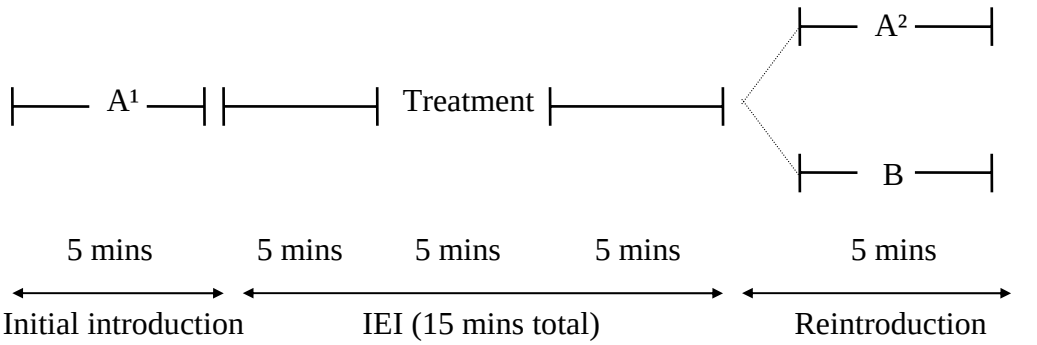
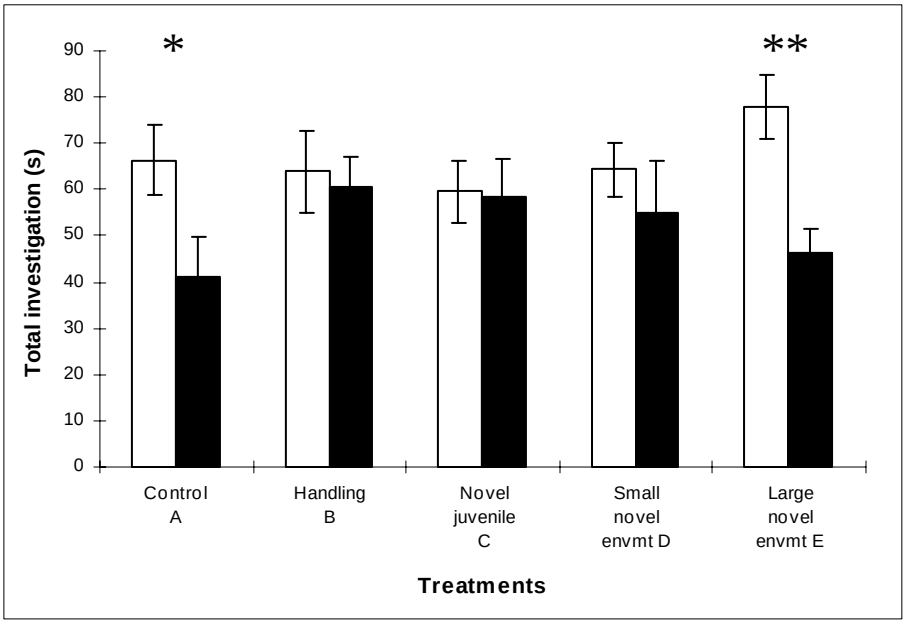


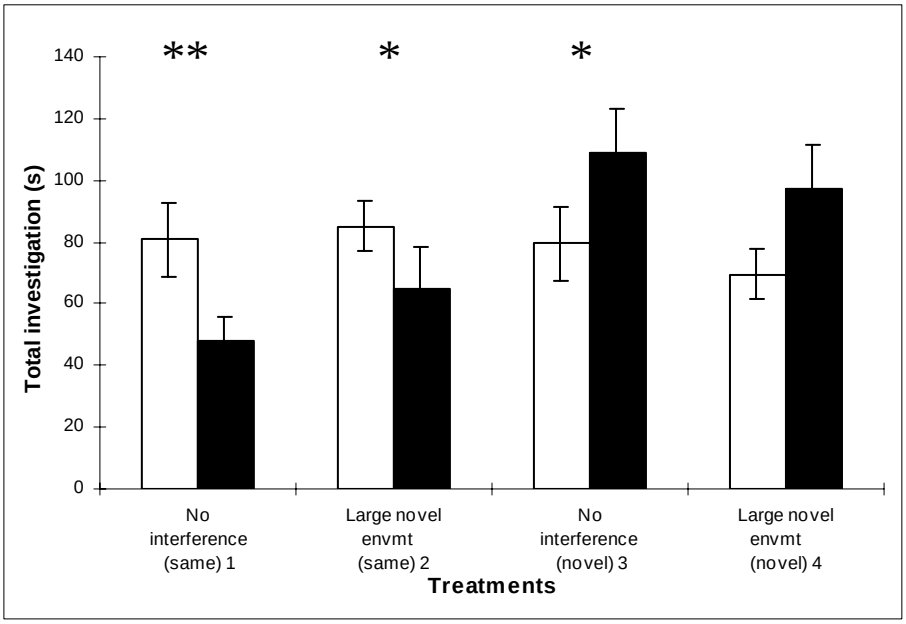
Figure Two:


*
**
90
80
70
60
50
40
30
20
10
0
Total investigation (s)
Control A
Handling B
Novel juvenile C
Small novel envmt D
Large novel envmt E
Treatments

582

Figure Four:

584

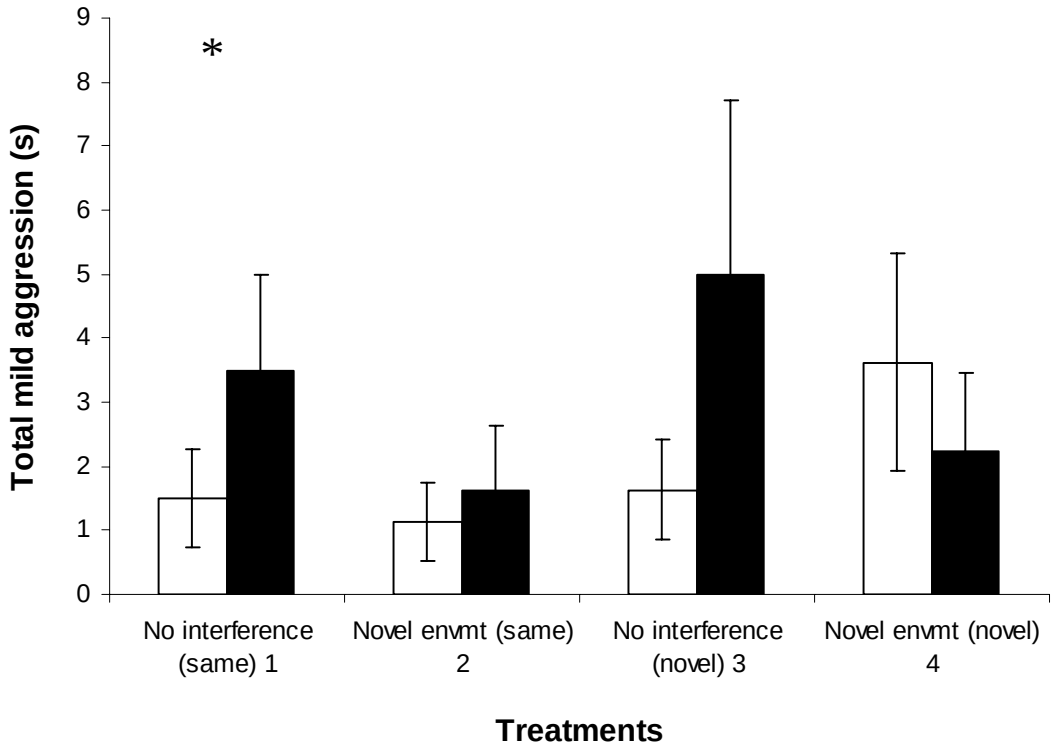



586

588

Figure Five:

590




592

594

596 Figure 1. The protocol for experiment one, with a disturbance treatment introduced midway through the inter-exposure interval, and the same juvenile stimuli
598 reintroduced for the second exposure.

600 Figure 2. Change in the total amount of investigation of the juvenile stimuli by the adult subjects between exposures, for five different treatments. The white columns
602 represent mean ± SE pre-treatment, the black columns represent mean ± SE post-treatment. * $P<0.05$; ** $P<0.01$

604

Figure 3. The protocol for experiment two, with a disturbance treatment introduced
606 midway through the inter-exposure interval, and either the same juvenile stimuli reintroduced, or a novel juvenile introduced, for the second exposure.

608

Figure 4. Change in the total amount of investigation of the juvenile stimuli by the
610 adult subjects between exposures, for four different treatments. The white columns represent mean ± SE pre-treatment, the black columns represent mean ± SE post-
612 treatment. * $P<0.05$; ** $P<0.01$

614 Figure 5. Change in the total amount of mild aggression directed towards the juvenile stimuli by the adult subjects between exposures, for four different treatments. The
616 white columns represent mean ± SE pre-treatment, the black columns represent mean ± SE post-treatment. * $P<0.05$